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Human biomonitoring in residents of a NORM area using the comet assay, Pernambuco-Brazil

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1. Introduction

Geological research has found areas of uraniferous phosphorite in the northeast of Brazil [1]. The presence of uranium in phosphate rock depends on the existence of the phosphate contained in it. To do this, uranium is deposited in the pores of phosphate minerals by means of chemical exchange of the calcium (Ca+2) present in them for uranium (U+4) [2]. The uranium-phosphate region is located along the coast of the states of Pernambuco and Paraíba, Brazil. Its distribution in Pernambuco is in the Recife Metropolitan Region (RMR), covering the municipalities of Paulista, Abreu e Lima and Olinda [3]. Studies have been carried out in these municipalities, verifying the presence of Naturally Occurring Radioactive Material (NORM) and finding radionuclides in the soil, groundwater and fruit [1, 4; 5]. This research led to this study using human biomonitoring (HBM) in residents of the municipalities of Olinda and Abreu e Lima who are under the influence of radionuclides in the uranium-phosphate region.

Human biomonitoring (HBM) can be defined as a method for assessing human exposure to chemical substances and their effects, by determining the concentrations of these substances, their metabolites and reaction products within the body. To do this, BHM involves the collection of biological samples in a number of matrices such as blood, urine and hair [6]. Blood is the most widely used matrix for internal exposure measurements, and can be used in the fraction of whole blood, plasma or serum [7; 8]. Blood cells are used in studies because they circulate throughout the body and their cellular, nuclear and metabolic states can reflect the exposure suffered by the organism. These include mononuclear cells such as the lymphocyte [9]. Cellular alterations are known as biomarkers and cytogenetic damage caused by their expression can be assessed using techniques such as the comet assay [10; 11].

The comet assay, also called single cell gel electrophoresis, consists of the electrophoresis of individual cells or nuclei in microgels and is used to detect and assess DNA damage. It can detect different types of DNA breaks, such as using single-cell alkaline gel electrophoresis to detect single strand breaks. After electrophoresis, the nuclei of the cells are stained with fluorescent reagents [12]. The results can be expressed in arbitrary units (AU) which correspond to the weighted sum of the comets read or in percentage damage (%) [13].

In view of the above, it is important to assess the genotoxicity of the population living in the regions where uraniferous phosphorite occurs in order to evaluate human biomonitoring and support the development of public health policies. This study aims to assess DNA damage in lymphocytes from residents of the municipalities of Olinda and Abreu e Lima using the alkaline comet assay. The research is highly relevant as it is a pioneer in generating scientific knowledge in this area of study.

2. Methodology

This study consisted of a sample group of volunteers living in the municipalities of Olinda (OP), Abreu e Lima (AL1, AL2, AL3 and AL4) and a control group of volunteers who did not live in the uranium-phosphate area (C1, C2 and C3), with a total of eight individuals analyzed. The selected volunteers were over 18 years old. The samples were only collected after signing the Free and Informed Consent Form (FICF) and answering the anamnesis questionnaire, making the volunteers suitable for the study. The exclusion criteria were active COVID-19 patients, having been exposed to ionizing radiation by treatment or diagnosis in the last 6 months, being a smoker, being a compulsive alcoholic and an oncology patient. A total of 4 ml of peripheral blood was collected from each individual through venipuncture with a syringe and sent to the Radiobiology Laboratory (LR) at the Federal University of

Pernambuco (UFPE). This study was approved by the Research Ethics Committee (CEP) involving the use of human beings at the Federal University of Pernambuco (no. 45611221.0.0000.5208 and substantiated opinion 4.709.099).

The alkaline comet assay was performed as described by SINGH et al., 1988 [14], with modifications. To obtain the lymphocytes, 2 ml of peripheral blood was aliquoted from each sample and mixed with 2 ml of phosphate-buffered saline (PBS) in a sterile 15 ml falcon tube and then centrifuged at 3,000 rpm for 15 min. It was then diluted in Ficoll® (1:2) and centrifuged once more using the same settings. A layer of lymphocytes was then formed in this homogenate. Around 100 µL of this layer was collected and homogenized in 100 µL of 0.5% low-melting agarose (Sigma-aldrich) dissolved in PBS at pH 7.4. Immediately, this new homogenate was placed on a microscope slide, previously covered with a layer of 1.5% normal melting point agarose (Sigma-aldrich), covered with a coverslip and kept at 4 °C for 10 minutes. The coverslips were then removed and the slides incubated in lysis solution (2.5 M NaCl, 100 mM EDTA, 10 mM Tris, 1% Triton-X 100 and 10% DMSO, pH 10.0) for 12 hours at 4 °C. After the lysis process, the slides were placed in a horizontal electrophoresis vat containing an alkaline buffer solution, pH 13.0 (EDTA 1 mM and NaOH 300 mM), for 20 minutes. Electrophoresis was then started for 20 min (4 °C) at 0.74 V/cm and 300 mA. At the end, the slides were neutralized with 0.4 M Tris buffer (pH 7.5) for 15 minutes and fixed with absolute alcohol for 10 minutes.

The slides were stained with 50 µL of a solution of SYBER safe (Invitrogen) (1:500 diluted in distilled water). 100 cells per individual were analyzed in triplicate under a fluorescence microscope (Nikon H550L) at 400x magnification. Visual analysis of DNA damage was carried out according to the methodology of Collins et al., 2008 [15]. The nuclei were divided into 5 categories of DNA damage (0 - 4), depending on the extent of the damage. Category 0 indicates that no damage has occurred, categories 1 to 4 indicate damage at increasing levels to the genetic material.

Genetic damage was assessed using two parameters: Damage Index (DI) and Damage Frequency (DF%). The DI was calculated by multiplying the level of damage by the number of comets corresponding to it, as described in Equation 1 below:

$$DI = 0 \times (n0) + 1 \times (n1) + 2 \times (n2) + 3 \times (n3) + 4 \times (n4) \quad (1)$$

The DF% was calculated as the percentage value of all comets with DNA damage (level 1 - 4) in relation to the total number of comets (level 0 - 4), according to Equation 2 below:

$$DF\% = \frac{[(total\ number\ of\ comets - total\ number\ of\ comets\ 0)\ x\ 100]}{total\ number\ of\ comets} \quad (2)$$

For the comet assay, the statistical models used were ANOVA and Bonferroni using the GraphPad Prism 5.0 statistical program, with $p < 0.05$ considered.

3. Results and Discussion

Figures 1 and 2 show the results of the Damage Index and Damage Frequency respectively. It was observed that there was no statistically significant difference between the sample and control groups in terms of Damage Index (DI) and Damage Frequency (DF%). Although the DI showed no statistically significant difference, it was observed that volunteer AL2 from the NORM area had the highest damage index (DI = 162.33 ± 14.47) compared to the other volunteers, with a large number of comets in grade four (20UA). In addition to this volunteer, volunteer AL4 from the NORM area also showed grade four comets (8UA). These individuals are not influenced by external factors such as smoking, compulsive alcohol consumption, advanced age or occupation [16]. Therefore, it is suggested that the damage presented may be related to exposure to uraniferous ore, present in the municipality of Abreu e Lima.

In Brazil, few studies on human biomonitoring in areas where uraniferous ore is present have been found in the literature. Among them, some also showed no statistically significant difference between their groups, such as the study by Guimarães et al. (2010) [17] which assessed the populations of the towns of Monte Alegre, Prainha and Alenquer in the state of Pará in a NORM area due to uranium mineralization.

Other studies involving human biomonitoring have also been carried out, such as Júnior et al. (2018) [18], who assessed the occupational exposure of miners in a coal mine in the city of Candiota, Rio Grande do Sul, and Muller et al. (2022) [19], who assessed occupational exposure to chromium in workers from electroplating companies in Rio Grande do Sul, which found a statistically significant difference in the Damage Frequency (DF%) when compared to the exposure-free group, something that does not corroborate the present study.

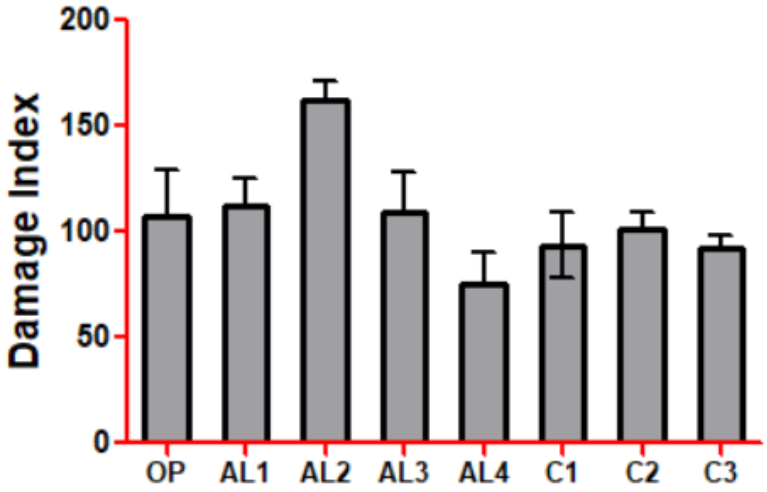

Figure 1: Damage index found in the comet assay.

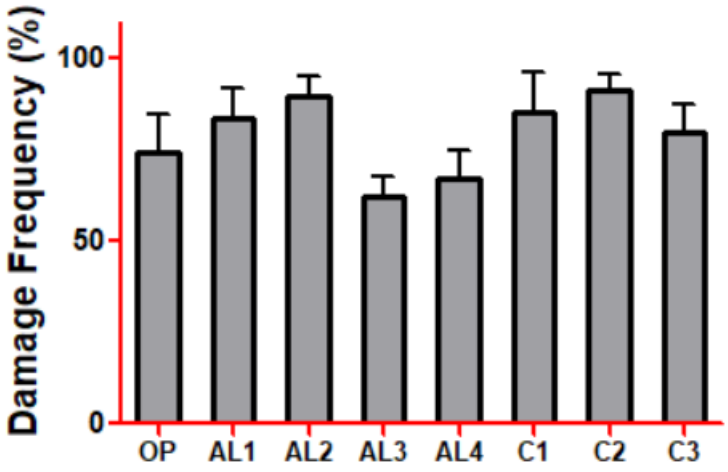

Figure 2: Damage frequency found in the comet assay.

4. Conclusions

The results showed that there was no statistically significant difference between the sample group and the control group in terms of damage index (DI) and damage frequency (FD%) present in human lymphocytes, but a large number of grade 4 comets were found in the blood samples of residents of the municipality of Abreu e Lima (AL2 and AL4). This suggests that this is due to natural exposure to the uraniferous ore present in this region.

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